REMARKS

Entry of the present amendments and consideration of the remarks which follow are respectfully requested by Applicants.

Claim 1 has been amended to eliminate the recitation of "m" and "L" in both the chemical structure and the text. No new matter has been added.

Claims 1, 3, 21, 22, 31, 33-37, 48, 49, 52, 54, 56, 59, 66, 81, and 82 are currently pending for examination.

Amendment of inventorship

Applicants again request amendment of inventorship due to amendment or cancellation of claims. Applicants request that the name of the person identified below be deleted from the list of inventors, and they acknowledge that the inventor's invention is no longer being claimed in this nonprovisional application. Please delete the following name:

Richard Terry Root

The Examiner did not approve Applicants' previous request for change of inventorship to delete Richard Terry Root as an inventor for the reason that the fee to charge their deposit account was not authorized by Applicants. That authorization, however, was expressly given just above the signature of the undersigned agent in Applicants' response filed on 11/1/06 and is herein repeated on the first and last pages of the present reply.

If there continues to be a question regarding authorization to charge the designated deposit account for the fee required under 37 CFR 1.17(i), the Examiner is invited to telephone the undersigned for authorization.

Rejection under 35 USC §112, second paragraph

Claims 1 and 3 have been rejected under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner argues that claim 1 is confusing and indefinite because the groups "m" and "L" remain defined as variables when "m" is defined as "0" and "L" is defined as "0 carbon atoms".

Applicants have now amended claim 1 to delete the recitation of the variables "m" and "L" both in the text and in the structure drawn, and they respectfully request the Examiner's reconsideration of the rejection.

Rejection under 35 USC §112, first paragraph

Claims 1 and 3 have again been rejected under 35 USC §112, first paragraph, for the reason stated in paragraph 6 of the Office Action mailed 5/8/06. The examiner argues that there is no enablement in the specification for the preparation of the compound of claim 1 where the linker "L" is absent (as required by the definition of "L").

Applicants traverse and argue that their specification does enable instances where "L" and "m" can be zero. An example where "m" is 0 is an ether linkage, which is described in Applicants' specification on page 13, paragraph [0054], line 6. Further, enablement for an instance where "L" is 0 and "m" is 0 is found on page 13, paragraph [0053], which teaches generating a compound according to paragraph [0065] where "L" is 0 and "m" is 0. For reference, a page is attached hereto showing the chemical pathways indicated in paragraph [0053]. The examiner's reconsideration of the rejection is respectfully requested by Applicants.

Rejection under 35 USC §103 (a)

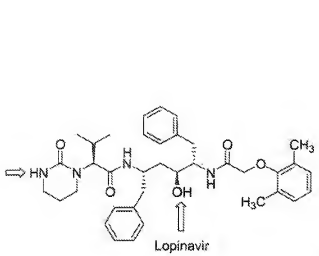
Claims 1, 3, 21, 22, 31, 33-37, 48, 49, 52, 54, 56, 59, 66, 81, and 82 have again been rejected under 35 USC §103 (a) as being unpatentable over the admitted prior art as set forth in the specification in combination with Vierling et al., FR 98 00728 (hereinafter "Vierling") and optionally with Bieniarz et al, US 5,380,873 (hereinafter "Bieniarz") for the reason set forth in paragraph 10 of the May 8, 2006 Office Action.

The Examiner was not persuaded by Applicants previous argument that there is no motivation to combine the prior art as set forth in Applicants' specification with that of Vierling and optionally with Bieniarz because the teaching of Vierling deals with prodrugs, i.e., drugs that are designed to be unstable in the body (i.e., to release the drug after ingestion by a patient).

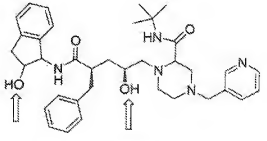
Applicants now further argue that the skilled artisan would not predict that the reactivity of a hydroxy function in one HIV protease inhibitor will necessarily be the same for a hydroxy group in another HIV protease inhibitor in the presence of other reactive functionalities, especially in the case of the HIV protease inhibitors lopinavir, saquinavir, and indinavir. There are significant structural

differences between lopinavir, the subject of the Applicants' invention, and the inhibitors saquinavir and indinavir used by Vierling, primarily on the ends of the molecules.

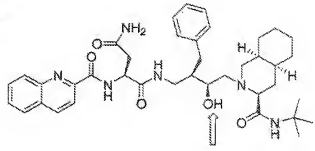
The inventors have seen, in an attempted acylation reaction targeting the central hydroxy group of lopinavir, under conditions that would be expected to give at least some product, reaction at the nitrogen at the tetrahydro-pyrimid-2-yl moiety. That this nitrogen is reactive has been shown by Stoner, E. et al., Organic Process Research & Development 4, 264-269, 2000 (copy attached hereto). The Stoner article would indicate to the person of ordinary skill in the art that the tetrahydro-pyrimid-2-yl moiety contains a reactive NH because intermediate 11 (Scheme 3, page 266), once formed, was unstable and gave rise to polymers. In order to get a polymer, there has to be another reactive group in the lopinavir precursor (compound 5), and by extension, also lopinavir. Vierling (Schéma 2, page 6) protected the reactive hydroxyl in indinavir using a well-known method, but Vierling did not teach what to do about the reactive NH group in lopinavir.



Lopinavir



Indinavir



Saquinavir

The disclosure of Bieniarz does not make up for the deficiencies of Vierling in this regard. Applicants argue that the reactivity of lopinavir cannot be predicted from that of saquinavir and indinavir

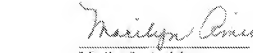
and that the Examiner's case for *prima facie* obviousness has thus not been made. They respectfully request the Examiner's reconsideration of the rejection under 35 USC §103(a).

Applicants submit that their application is now in condition for allowance, and favorable reconsideration of their application in light of the above amendments and remarks is respectfully requested. Allowance of claims 1, 3, 21, 22, 31, 33-37, 48, 49, 52, 54, 56, 59, 66, 81, and 82 at an early date is earnestly solicited.

* * * * * *

The examiner is hereby authorized to charge any fees associated with this Amendment to Deposit Account No. 02-2958. A duplicate copy of this sheet is enclosed.

Respectfully submitted,



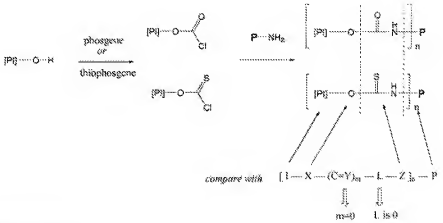
Marilyn L. Amick
Reg. No. 30,444

Customer no. 23690
Phone: 317-521-7561

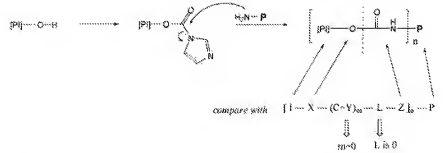
Chemical reactions referred to in specification, page 13, paragraph [0053]

[0053] Yet another approach for generating urethane, urea and thiourea bonds at the point of attachment to the HIV protease inhibitor is to first treat the target hydroxyl or amine function with phosgene or thiophosgene to give an oxycarbonyl chloride or oxythiocarbonyl chloride. The latter intermediates react readily with amines to give urethanes, ureas or thioureas. Alternative phosgene equivalents such as carbonyldiimidazole or disuccinimidyl-carbonate will react similarly.

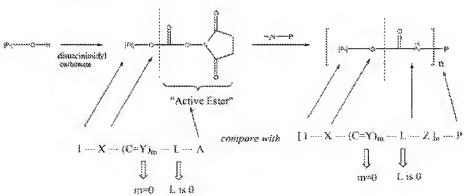
Treating target hydroxyl with phosgene or thiophosgene gives an oxycarbonyl chloride or oxythiocarbonyl chloride. The latter intermediates react readily with amines to give urethanes, ureas, or thioureas:



Using carbonyldiimidazole:



Using disuccinimidyl-carbonate:



Organic Process Research & Development 2000, 4, 264–269

Synthesis of HIV Protease Inhibitor ABT-378 (Lopinavir)

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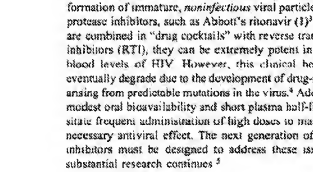
Abbott Laboratories, 1401 Sheridan Road, North Chicago, Illinois 60064-6327, U.S.A.

Abstract:

A large scale process for the synthesis of HIV protease inhibitor candidate ABT-378 has been developed which utilizes an intermediate common to the synthesis of ritonavir, Abbott's first generation compound. The synthesis relies on the sequential acylation of this intermediate which is carried through as a mixture of diastereomers until the penultimate step. A synthesis of acid 5, derived from L-valine, is also reported.

Introduction

The approval of the first HIV-protease inhibitors in early 1996 provided the world with powerful new weapons in the fight against HIV, the virus responsible for AIDS.[1] HIV-protease is an enzyme critical to the life-cycle of the virus, and its inhibition disrupts viral replication, resulting in the formation of immature, *noninfectious* viral particles.[2] When protease inhibitors, such as Abbott's ritonavir (**1**)[3] (Norvir), are combined in "drug cocktails" with reverse transcriptase inhibitors (RTI), they can be extremely potent in reducing blood levels of HIV. However, this clinical benefit can eventually degrade due to the development of drug-resistance arising from preexistent mutations in the virus.[4] Additionally, modest oral bioavailability and short plasma half-life necessitate frequent administration of high doses to maintain the necessary antiviral effect. The next generation of protease inhibitors must be designed to address these issues, and substantial research continues.[5]



(1) Deeks, S. G.; Smith, M.; Holodney, M.; Kahn, J. O. *J. Am. Med. Assoc.* **1997**, *277(3)*, 145.

(2) Moyle, G.; Gazzard, B. *Drugs* **1996**, *51(3)*, 70.

(3) Also referred to as ABT-538, see: Kempf, D. J.; Marsh, K. C.; Denissen, J. F.; McDonald, E.; Vasavanonda, S.; Flentge, C. A.; Green, B. E.; Fino, L.; Park, C. H.; Kong, X.-P.; Wideburg, N. E.; Saldivar, A.; Ruiz, L.; Kati, W. M.; Sham, H. L.; Robins, T.; Stewart, K. D.; Hsu, A.; Plattner, J. J.; Leonard, J. M.; Norbeck, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92(7)*, 2484.

(4) (a) Molla, A.; Korneyeva, M.; Gao, Q.; Vasavanonda, S.; Schipper, P. J.; Mo, H.-M.; Markowitz, M.; Chernyavskiy, T.; Niu, P.; Lyons, N.; Hsu, A.; Granneman, G. R.; Ho, D. D.; Boucher, C. A. B.; Leonard, J. M.; Norbeck, D. W.; Kempf, D. J. *Nat. Med.* **1996**, *2(7)*, 760. (b) Schinazi, R.-C.; Ruiz, L.; Cloret, B.; Raventos, A.; Toy, J.; Leonard, J.; Desmyter, J.; De Clercq, E.; Vandamme, A.-M. *AIDS* **1996**, *10*, 995.

Promising reports of Abbott's next generation protease inhibitor candidate, ABT-378 (**2**, lopinavir), have recently appeared.[6,7] This compound, when co-administered with much smaller doses of ritonavir (**1**), shows substantially better bioavailability and activity against wild-strain HIV-1 and certain mutations than ritonavir alone. Additionally, high plasma levels of ABT-378 can be maintained with much smaller doses of drug, potentially obviating many of the side effects that compromise adherence by a patient to a treatment regimen.[6]

The rapid development of ABT-378 (**2**) required the preparation of significant quantities of unformulated "bulk" drug. Therefore, it was critical for us to quickly discover, develop, and *implement* an efficient, high-yielding, and cost-effective synthesis. The structural similarities between ritonavir (**1**) and ABT-378 (**2**) allowed us to take advantage not only of earlier process research but also to potentially utilize certain common synthetic intermediates as well.

Retrosynthesis of ABT-378 (2)

Our general synthetic strategy is similar to that employed for ritonavir in which the "core" protected diamino alcohol **4** is acylated sequentially with the side chain acids **3**[a] and **5** (Scheme 1).

Protected diamino alcohol **4** is readily available in quantity from ritonavir manufacturing.[10] In that process, L-phenylalanine is sequentially tribenzylated, treated with acetonatrile anion to produce a cyanomethylketone, and subsequently exposed to benzyl magnesium chloride, producing an enantiomer in >99% ee.[10a] Stepwise-reduction of this species

(5) Sham, H. L.; Chen, X. *Exp. Opin. Invest. Drugs* **1996**, *5(8)*, 977.

(6) Sham, H. L.; Kempf, D. L.; Molla, A.; Marsh, K. C.; Kumar, G. N.; Chen, C.-M.; Kati, W.; Stewart, K.; Lal, R.; Hsu, A.; Betebenner, D.; Korneyeva, M.; Vasavanonda, S.; McDonald, E.; Saldivar, A.; Wideburg, N.; Chen, X.; Niu, P.; Park, C.; Jayanti, V.; Grabowski, B.; Granneman, G. R.; Sun, E.; Japour, A. J.; Leonard, J. M.; Plattner, J. J.; Norbeck, D. W. *Antimicrob. Agents Chemother.* **1998**, *42*, 3218.

(7) Sham, H. L.; Norbeck, D. W.; Chen, X.; Betebenner, D. A.; Kempf, D. J.; Herrin, T. R.; Kumar, G. N.; Condon, S. L.; Cooper, A. J.; Dickman, D. A.; Hannick, S. M.; Kolaczkowski, L.; Oliver, P. A.; Plata, D. J.; Stengel, P. J.; Stoner, E. J.; Tien, J.-H. J.; Liu, J.-H.; Patel, K. M. U.S. Patent Application 98-57236; *Chem. Abstr.* **1997**, *127*: 122901.

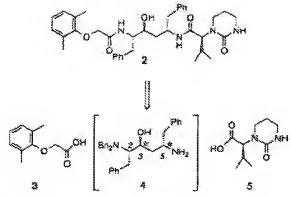
(8) (a) Molla, A.; Vasavanonda, S.; Kumar, G. N.; Sham, H. L.; Johnson, M.; Grabowski, B.; Denissen, J. F.; Kohlbrenner, W.; Plattner, J. J.; Leonard, J. M.; Norbeck, D. W.; Kempf, D. J. *Virology* **1998**, *250*, 255. (b) Carillo, A.; Stewart, K.; Sham, H.; Norbeck, D.; Kempf, D.; Kohlbrenner, W.; Leonard, J.; Molla, A. *J. Virology* **1998**, *72*, 7532.

(9) Hayes, N. V.; Branch, G. E. K. *J. Am. Chem. Soc.* **1943**, *65*, 1555.

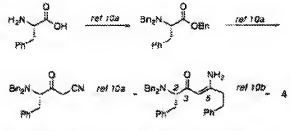
(10) (a) Stek, T. L.; Haight, A. R.; Scarpetti, G.; Allen, M. S.; Menzia, J. A.; Robbins, T. A.; Parekh, S. I.; Langridge, D. C.; Tien, J.-H. J.; Patrick, R. J.; Kerdesky, F. A. *J. J. Org. Chem.* **1994**, *59*, 4040. (b) Haight, A. R.; Stek, T. L.; Allen, M. S.; Bhagavatula, L.; Fitzgerald, M.; Hannick, S. M.; Kerdesky, F. A. J.; Menzia, J. A.; Parekh, S. I.; Robbins, T. A.; Scarpetti, D.; Tien, J.-H. J. *Org. Process Res. Dev.* **1999**, *3*, 94. (c) Haight, A. R.; Stek, T. L.; Menzia, J. A.; Robbins, T. A. *Tetrahedron Lett.* **1997**, *38(24)*, 4191.

Published on Web 05/27/2000
10.1021/op9902628 CCC: $19.00

Scheme 1



Scheme 2

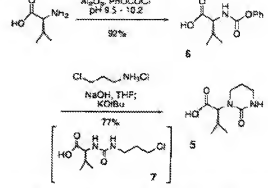


produces 4.[10a] These transformations yield 4 as a mixture of diastereomers in which the desired isomer (*2S,3S,5S*) typically comprises 89−93% of the whole with the remainder being the three undesired diastereomers.[11] In our synthesis of **2**, this diastereomeric mixture is used without further purification (Scheme 2).

Synthesis of Acid 5

With the "core" of the molecule (**4**) in hand, the next priority was to develop an efficient synthesis of each of the side chains. We were fortunate that acid **3** derived from 2,6-dimethylphenol was available by a modification of a known procedure.[12] The preparation of **5**, however, required more effort.

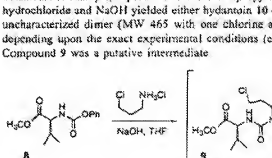
The original medicinal route to **5** was a low-yielding six-step synthesis starting with 3-aminopropanol and L-valine methyl ester hydrochloride salt. In our improved synthesis, L-valine was first converted to *N*-phennxycarbonyl-L-valine **6**[13] with phenylchloroformate. Previously, reported syntheses of **6** were found to be cumbersome on large scale and were modified.[14] For example, LiCl was added to provide a lower freezing point to the aqueous solution which provided better control of the reaction. Additionally, LiOH was found to be a superior base to all others employed. Neutral Al_2O_3 was used to prevent gumming and emulsion formation during the course of the reaction which aided in accurate pH monitoring. Control of pH was essential as valine dimer (val-val dipeptide) and its acylated derivatives were formed as significant reaction byproducts outside of this optimized pH window.



Treatment of **6** with 3-chloropropylamine hydrochloride and solid NaOH in THF affords the unisolated salt of chloropropylurea **7** (eq 1) While both NaOH and LiOH facilitate this reaction, KOH appears to cause degradation of **6** and is unsuitable. Crude **7** is then treated with KOtBu effecting cyclization to the desired acid **5**. The product **5** is isolated as a nearly colorless solid in 75−85% yield and in >99% ee.

The quality of the 3-chloropropylamine hydrochloride used is critical. Commercially prepared material frequently contains dark colored impurities which are difficult to remove and carry through the entire synthesis: the use of high quality amine is essential.[13]

Subsequently, we explored the above chemistry using L-valine methyl ester. Unfortunately, when this two-step protocol was applied to the ester, very different results were obtained. For example, exposure of **8** to 3-chloropropylamine hydrochloride and NaOH yielded either hydantoin **10** or an uncharacterized dimer (MW 465 with one chlorine atom) depending upon the exact experimental conditions (eq 2). Compound **9** was a putative intermediate



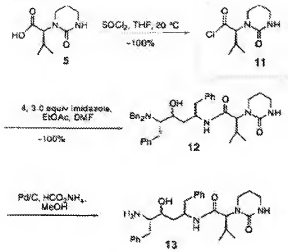
(11) While the % de of **4** is determined, the % ee is not measured. Subsequent processing in the ritonavir synthesis incorporates one additional chiral center, the enantiomer of **4** would be manifested as a ritonavir diastereomer, which is not observed. This suggests that the % ee of **4** is a reflection of the precursor enantiomer.

(12) *Organic Syntheses*; Baumgarten, H. E., Ed.; Wiley: New York, 1973; Collect. Vol. 5, p 251.

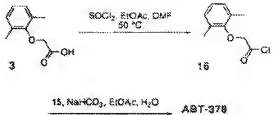
(13) This compound is used in the manufacturing of ritonavir as well

(14) Wuts, P. G. M.; Pruett, L. E. *Synthesis* **1989**, 622.

Scheme 3



Scheme 4



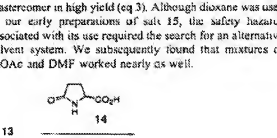
Synthesis of ABT-378

Acylation of 4 with acid 5 was initially achieved by well-known peptide coupling methods. Optimization of this transformation was investigated in order to discover a more cost-effective method.

We first examined the preparation of activated derivatives of 5, but the neutral amides were impeded by the instability of these derivatives. For example, exposure of 5 to oxalyl chloride or isobutyl chloroformate under standard protocols resulted in the formation of an intractable polymer rather than the desired acyl chloride 11. Attempts to trap the intermediate anhydrides in situ as activated esters were also unsuccessful. We were fortunate to find that thionyl chloride or phosphorus oxychloride provided 11 (ironically, a stable solid) in near quantitative yield without decomposition.[16] Acyl chloride 11 is only sparingly soluble in THF; once prepared it can be dissolved in DMF and used immediately. A large number of bases were screened for the coupling of 11 with 4, and imidazole was found to be superior.[17]

The reaction of dibenzylamino alcohols 4 with acyl chloride 11 in the presence of 3.0 equiv of imidazole in EtOAc and DMF afforded monoacylated intermediate 12 as a mixture of diastereomers.[16] This mixture was carried on after workup without any further purification and was subjected to debenzylation with Pd/C and HCO_2NH_4 in MeOH at 50 °C (Scheme 3). The debenzylation leading to 13 was clean and without significant complications.[18] We were able to achieve similar debenzylations with Pd/C in the presence of formic acid or hydrogen gas; however, the protocol described was the most reliable and amenable to large scale synthesis. As expected, the acylation and debenzylations leading to 13 had no appreciable effect on the ratio of diastereomers. Amine 13 was identified as the most logical point for improving the diastereomeric purity.

Extensive screening on the purification of 13 by the formation of a diastereomeric salt yielded a practical procedure. More than 50 acids were screened in a variety of solvents before one was found which gave the desired purification combined with a high recovery of an isolable solid. Exposure of crude 13 to *S*-2-pyrrolidone-5-carboxylic acid (L-pyroglutamic acid) (14) in dioxane at 50 °C followed by cooling, allowed for the isolation of 15 as virtually a single diastereomer in high yield (eq 3). Although dioxane was used in our early preparations of salt 15, the safety hazards associated with its use required the search for an alternative solvent system. We subsequently found that mixtures of EtOAc and DMF worked nearly as well.



With pure 15 in hand, the second acylation was undertaken. In analogy to our earlier acylation experiments with 11, we found the simplest approach was the most effective, and therefore acyl chloride 16 was prepared from acid 3. Exposure of salt 15 to acyl chloride 16 under heterogeneous (Schotten−Baumann) reaction conditions in the presence of $NaHCO_3$, afforded 2 in high yield and purity. The only significant impurities in the crude acylation mixtures were identified as multiply acylated derivatives present in trace quantities only (Scheme 4).

Although pure ABT-378 (2) can be obtained by recrystallization from mixtures of ethyl acetate in heptane, small amounts of solvent are retained in the isolated solid. Removal of the final traces of solvent proved exceedingly difficult and even extensive drying after milling (to reduce particle

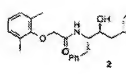
(15) High quality 3-chloropropylamine hydrochloride can be obtained by carbon treatment of commercial materials followed by recrystallization or by synthesis from 3-aminopropanol.

(16) A more detailed discussion of the optimization of this reaction is available: Sorter, E. J.; Cooper, A. J.; Stengel, P. J. *Org. Process Res. Dev.* **1999**, *3*, 145.

(17) Racemization of acyl chloride 11 during coupling is observed only when the material is allowed to stand in solution at room temperature for extended periods of time (several hours). If prepared and used immediately, no racemization is detected as the formation of the known (and independently prepared) diastereomer (17) (see ref 20).

(18) We did infrequently observe some catalyst poisoning in the debenzylation which we attributed to sulfur-based impurities carried through from the use of SOCl2 in the previous step. Although this necessitated a second charge of catalyst to effect complete reaction, it did not otherwise affect the process.

Scheme 5



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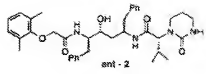

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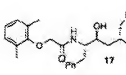
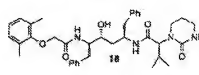


17



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size) did not facilitate its complete removal.[19] A secondary isolation procedure has been developed in which crystalline **2** is first dissolved in ethanol and then added slowly to a significantly larger volume of rapidly stirring water. The resulting slurry of partially amorphous **2** is isolated by filtration. This solid is dried virtually solvent free and is acceptable from a formulations standpoint.

By this four-step procedure, ABT-378 is produced in 58% overall yield from diamine alcohols **4**. Utilizing commonly available reagents and robust reaction conditions, this process is amenable to large-scale production and has been used to prepare multi-kilogram quantities of ABT-378 (**2**) in >99% de (see Scheme 5).[20]

Experimental Section

Melting points were measured with a capillary apparatus and are uncorrected. All IR spectra were measured from KBr pellets. ^{1}H NMR spectra were taken in $CDCl_3$ unless otherwise mentioned with $CHCl_3$ (7.26 ppm) used as an internal standard. ^{13}C NMR were taken in $CDCl_3$ unless otherwise mentioned, with $CDCl_3$ (77.00 ppm) used as an internal standard. All reactions were performed under a positive pressure of nitrogen. Solvent concentration was accomplished by rotary evaporation ~20 mmHg, with the bath temperature never exceeding 45 °C. Commercial grade anhydrous solvents and reagents were used without further purification unless otherwise specified. Unless otherwise specified all reactions were monitored by HPLC with purities being determined by peak area % at 205 nm. Optical rotations and microanalyses were performed by Robertson Microlit Labs. In the case of compounds which were isolated as mixtures of diastereomers, the spectral data presented represents the major, desired isomer.

***N*-Phenoxycarbonyl-L-valine (6).** L-Valine (100.0 g, 0.854 mol, 1 equiv), LiCl (60.0 g, 1.42 mol, 1.66 equiv), neutral aluminum oxide (32.0 g, 150 mesh), and 600 mL of water were charged to a suitable reaction vessel and cooled to −14 °C. The vessel was fitted with a pH probe, and the cooled suspension was adjusted to pH 10 using a 3.2 *M* solution of LiOH. Phenylchloroformate (140.4 g, 0.896 mol, 1.05 equiv) precooled to −20 °C was added. Additional 3.2 *M* solution of LiOH was added slowly to maintain the pH between 9.8 and 10.0 during the reaction. During the course of the addition, the reaction temperature was maintained at below −10 °C and the addition of LiOH solution maintained until the pH remained constant and the reaction complete. Control of pH is critical to avoid the formation of dimeric species.

After 5 h, the pH stabilized, and all of the starting material had been consumed. The white suspension was then filtered, and the collected solids were washed with 160 mL of water. The aqueous phases were collected and washed with 320 mL of methyl-*tert*-butyl ether. Toluene (800 mL) was added to the aqueous layer which was neutralized to pH ≈ 2 with concentrated H_2SO_4. The organic layer was separated and concentrated in vacuo at below 50 °C. The residue was dissolved in 300 mL of toluene at 40 °C, filtered, and treated with 240 mL of heptanes. The product crystallized from this solution after cooling to 0 °C and was collected by filtration after washing with 160 mL of 1:1 (v/v) toluene/heptane. The wet filter cake was dried in vacuo affording 188.1 g (93%) of **6** as a colorless solid (>99.5% purity by HPLC).

***(S)*-Tetrahydro-α-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetic acid (5).** A suitable flask was charged with *N*-phenoxycarbonyl-L-valine (**6**, 100.0 g, 0.42 mol, 1 equiv), 3-chloropropylamine hydrochloride (60.5 g, 0.47 mol, 1.12 equiv), and 1000 mL of THF and cooled to 2 °C. Solid NaOH (50.7 g, 1.27 mol, 3.02 equiv) was added to the stirring suspension. The reaction was stirred at less than 10 °C until the valine derivative was completely consumed by HPLC (about 2 h).

A solution of KOtBu (118.4 g, 1.06 mol, 2.51 equiv) in 300 mL of THF was added to the reaction mixture over 15 min and the internal temperature of the reaction allowed to rise to 20 °C. Stirring was continued at room temperature until the cyclization was complete (about 18 h).

The reaction mixture was quenched with 800 mL of distilled water and acidified to pH 9 with concentrated aqueous HCl (~100 g) while keeping the temperature below 30 °C. The aqueous layer was separated and 250 mL of ethanol added. This aqueous layer was then brought to pH 3 with concentrated HCl and extracted twice with ethyl

(19) Crystallographic studies have shown, to our surprise, that **2** isolated by this crystallization method is not a solvate.

(20) The determination of the enantiomeric excess (% ee) for ABT-378 (**2**) can be done indirectly. Compound **17**, which results from the acylation of **4** with the enantiomer of acid **5**, is known to us, having been detected as an impurity in our process development.[12] Compound **18** can only result from the acylation of the enantiomer of **4** (*2R,3R,5R*) with **5**. The levels of **17**/**18** observed in **2** are typically <0.1%. Until there is a need for a more definitive assay, we assume this represents an upper limit to the amount of ent-**2** present.

acetate (1000 mL and 400 mL). The combined organic layers were evaporated to dryness in vacuo.

The residual solid was dissolved in 600 mL of anhydrous 3A ethanol at reflux, carbon-treated to remove color, filtered, and reduced to dryness in vacuo. The residue was dissolved in 600 mL of hot ethyl acetate. Approximately one-third of the total volume was removed by atmospheric distillation and the suspension cooled to below 10 °C for 1 h. The product was isolated by filtration and dried in vacuo at less than 45 °C, affording 64.6 g of an off-white solid (77%) in >99% ee.[21]

IR: 3300, 2960, 2500 (br, weak), 1930 (br, weak), 1720, 1618, 1535, 1320, 1290 cm^{-1}. ^{1}H NMR (400 MHz, d_6-DMSO): δ 12.40 (br s, 1H), 6.18 (br s, 1H), 4.41 (d, J = 10 Hz, 1H), 3.25 (m, 1H), 3.14 (m, 1H), 3.08 (app dt, 2H), 2.08 (m, 1H), 1.87 (app p, 2H), 0.92 (d, J-7 Hz, 3H), 0.84 (d, J = 7 Hz, 3H). ^{13}C NMR (100 MHz, d_6-DMSO): δ 173.1, 155.8, 61.8, 41.7, 39.5, 26.6, 21.8, 19.8, 19.0.). MS (DCI/NH$_3$): 201 (M + H)$^+$, 218 (M + NH$_4$)$^+$. Anal. Calcd for $C_8H_{16}N_2O_2$: C, 53.99; H, 8.05; N 13.99. Found: C, 54.00; H, 7.96; N 14.11

[1*S*-[1*R(*R**),3*R**,4*R**]]-*N*-[4-[bis(phenylmethyl)amino]-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]tetrahydro-α-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetamide (12).** To a suitable flask equipped with mechanical stirring was charged acid **5** (35.2 g, 0.176 mol, 1.02 equiv) and 480 mL of THF. The resulting suspension was cooled to 4 °C, and thionyl chloride (28.6 g, 0.240 mol, 1.37 equiv) was added dropwise over 10 min. The resulting thick slurry was warmed to room temperature and stirred for 5.5 h, at which time HPLC revealed complete consumption of the acid.[22] The reaction mixture was reduced to dryness in vacuo. Heptane (250 mL) was added to the residue and the slurry again reduced to dryness in vacuo. The residual solid acyl chloride **11** was then partially dissolved in 170 mL of dry DMF.

Ethyl acetate (50 mL) and 80.0 g (0.172 mol, 1.0 equiv) of **4** (combined HPLC purity of the 4 diastereomers is >93% with the desired isomer present in 87%) were charged to a mechanically stirred reaction vessel. The solution was cooled to 2 °C, and 36.0 g (0.529 mol, 3.07 equiv) imidazole was added. To this reaction mixture was rapidly added the slurry of **11** prepared above. The reaction mixture was stirred at 4 °C for 1 h and subsequently warmed to 30 °C overnight. The reaction mixture was then quenched with a solution of 32.5 g of concentrated aqueous HCl in 260 mL of water, which, after mixing, made the lower aqueous layer pH = 3. After the solution was mixed for 30 min, the organic layer was separated and washed three times with 250 mL of saturated NaCl solution. The organic layer was then evaporated to dryness in vacuo, producing 96.4 g of **12** as a foamy off-white solid (87% yield). Crude **12** assays as >94% pure by HPLC (combined total of 4 diastereomers).

IR: 3381 (br), 3060, 3026, 2950, 2932, 2869, 1948 (w), 1874 (w), 1797 (w), 1643 (st), 1509, 1496, 1452, 1307, 748, 699 cm^{-1}. ^{1}H NMR (400 MHz): δ 7.08–7.37 (m, 20H), 6.76 (app d, J = 7.6 Hz, 1H), 4.94 (br s, 1H), 4.29 (app d, J = 10.8 Hz, 1H), 4.07–4.21 (m, 1H), 3.96 (app d, J = 13.2 Hz, 2H), 3.61 (dt, J = 2.4, 8.0 Hz, 1H), 3.39 (d, J = 13.6 Hz, 2H), 3.01–3.15(m, 4H), 2.93–2.99 (m, 1H), 2.60–2.82 (m, 5H), 2.15–2.24 (m, 1H), 1.67–1.75 (m, 1H), 1.53–1.66 (app ddt, 2H), 1.51 (sept, J = 7.6 Hz, 1H), 0.87 (app d, J = 5.6 Hz, 3H), 0.84 (app d, J = 5.6 Hz, 3H). ^{13}C NMR (100 MHz): δ 170.4, 156.7, 140.5, 139.3, 138.6, 129.4, 129.3, 129.1, 128.6, 128.5, 128.2, 127.2, 126.2, 126.0, 69.1 (CH), 64.0 (CH), 63.0 (br, CH), 54.1 (CH$_2$), 49.3 (CH), 41.1 (CH$_2$), 40.8 (br, CH$_2$), 40.2 (CH$_2$), 39.8 (CH$_2$), 31.7 (CH$_2$), 25.3 (CH), 21.6 (CH$_3$), 19.6 (CH$_3$), 18.7 (CH$_3$). MS (DCI/NH$_3$) 647 (M + H)$^+$. Anal. Calcd for $C_{41}H_{50}N_4O_3$: C, 76.12; H, 7.79; N 8.66. Found: C, 75.92; H, 7.84; N 8.63.

[1*S*-[1*R(*R**),3*R**,4*R**]]-*N*-[4-amino-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]tetrahydro-α-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetamide (13).** A 1-L, three-necked, round-bottomed flask equipped with a mechanical stirrer, reflux condenser with nitrogen inlet adapter, and a thermometer was charged with 55.7 g of crude **12** (0.086 mol, 1.0 equiv), 15.1 g of ammonium formate (0.239 mol, 2.78 equiv), 10.4 g of 5% w/w palladium on carbon (50% wet), and 260 mL of methanol. The reaction mixture was heated to 50 °C overnight at which time HPLC analysis revealed complete consumption of the starting material. The reaction mixture was filtered through a pad of diatomaceous earth which was washed once with 250 mL of methanol. The combined filtrates were reduced to dryness affording 41.9 g of a yellow syrup (104%).

IR 3315, 3060, 3027, 2961, 2869, 1643 (st), 1509, 1452, 1307, 701 cm^{-1}. ^{1}H NMR (400 MHz): δ 7.14–7.31 (m, 10H), 5.21 (br s, 1H), 4.11–4.35 (m, 2H), 3.54–3.58 (app sextet, J ~ 4 Hz, 1H), 3.15–3.25 (br m, 2H), 3.05–3.14 (m, 1H), 2.91–3.01 (m, 2H), 2.61–2.90 (m, 4H), 2.53 (dd, J = 9.6, 13.6 Hz, 1H), 2.15–2.24 (m, 2H), 1.71–1.85 (M, 3H), 1.53–1.64 (M, 1H), 1.40–1.51 (m, 1H), 1.31 (d, J = 16.8 Hz, 1H), 0.91 (d, J = 6.4 Hz, 3H), 0.84 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz): δ 170.5, 156.8, 139.2, 138.6, 129.5, 129.4, 129.1, 128.7, 128.6, 128.4, 128.3, 126.4, 126.1, 71.5 (CH), 64.6 (CH), 56.3 (CH), 48.6 (CH), 41.5 (CH$_2$), 40.8 (CH$_2$), 40.0 (CH$_2$), 39.5 (CH$_2$), 38.9 (CH$_2$), 25.3 (CH), 21.6 (CH$_3$), 19.6 (CH$_3$), 18.5 (CH$_3$). MS (ESI+) 467 (M + H)$^+$, 489 (M + Na)$^+$. MS (ESI$^-$) 465 (M − H)$^-$. Anal. Calcd for $C_{27}H_{38}N_4O_3$: C, 69.50; H, 8.21; N 12.01. Found: C, 69.58; H, 8.12; N 11.65.

[1*S*-[1*R(*R**),3*R**,4*R**]]-*N*-[4-amino-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]tetrahydro-α-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetamide, 5-Oxo-L-proline salt (15).** Crude **13** (37.3 g, 0.080 mol, 1 equiv) was slurried in 150 mL of 1,4-dioxane at room temperature. The dioxane is subsequently removed in vacuo, and 370 mL of dioxane was charged to the flask.[23] Solid L-pyroglutamic acid (**14**) (10.3 g, 0.080 mol, 1 equiv) was added and the suspension heated to 50 °C, which resulted in the formation of a clear, yellow-colored solution. After 1 h at 50 °C, no solid was present, and the solution was slowly cooled to room temperature

(21) Enantiomeric excess is determined by HPLC (Chiralcel OD column, elution with hexane/ethanol/trifluoroacetic acid (930/70/1). The desired L-isomer has a retention time of approximately 14 min; the D-isomer, 11.5 min.

(22) Analysis was performed on crude reaction aliquots quenched into methanol.

(23) Karl Fischer titration at this point: 0.07% water. Anhydrous conditions facilitate the crystallization.

overnight. During this time the solid product **15** precipitated out and was subsequently collected by filtration. The solid product was washed with 100 mL of dioxane and dried in vacuo at 60 °C with a strong N_2 purge to afford 35.2 g of colorless **15** (74% yield). The solid assays as >98.5% pure by HPLC; a small amount of dioxane is also present.

IR: 3400 (br), 3061, 3022, 2962, 2867, 1659, 1586, 1512, 1452, 1306, 701 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ 7.12−7.38 (M, 10H), 4.23−4.28 (m, 1H), 4.19 (d, J = 11 Hz, 1H), 4.05 (dd, J = 6.0, 8.4 Hz, 1H), 3.72−3.77 (m, 1H), 3.49 (app dt, J = 3.6, 7.6 Hz, 1H), 3.10−3.16 (m, 2H), 3.00−3.09 (m, 2H), 2.89−2.98 (m, 2H), 2.71−2.76 (m, 1H), 2.53 (dd, J = 10.0, 13.6 Hz, 1H), 2.33−2.49 (m, 1H), 2.26−2.32 (m, 2H), 2.03−2.12 (m, 2H), 1.71−1.83 (m, 2H), 1.63−1.70 (m, 1H), 1.45−1.51 (m, 1H), 0.79 (d, J = 6.8 Hz, 3H), 0.76 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz): δ 181.4, 180.0, 171.9, 158.7, 139.7, 137.5, 130.71, 130.69, 130.3, 129.6, 128.6, 127.4, 68.1 (CH), 63.9 (CH), 59.6 (CH), 57.7 (CH), 48.6 (CH), 41.6 (CH_2), 41.0 (CH_2), 40.7 (CH_2), 40.69 (CH_2), 37.9 (CH_2), 34.1 (CH_2), 26.9 (CH_2), 26.8 (CH), 22.4 (CH_2), 20.0 (CH_3), 18.8 (CH_3). MS (ESI+) 467 (M + H)$^+$, 489 (M + Na)$^+$. MS (ESI−) 465 (M − H)$^-$. Anal. Calcd for $C_{23}H_{48}N_2O_6$: C, 64.52; H, 7.61; N 11.76. Found: C, 64.54; H, 7.70; N 11.54.

[1*S*-[1*R(*R**),3*R**,4*R**]]-*N*-[4-[[(2,6-dimethylphenoxy)-acetyl]amino]-3-hydroxy-5-phenyl-1-(phenylmethyl)pentyl]tetrahydro-α-(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetamide (2).** Acyl chloride **16** was prepared by the reaction of 7.26 g acid **3** (0.0403 mol, 1.2 equiv), 22 mL of EtOAc, and 5.75 g of thionyl chloride (0.0483 mol, 1.4 equiv) at room temperature. A single drop of DMF was added, and the slurry was warmed to 50 °C, eventually affording a clear solution after 5 h. The solution containing acyl chloride **16** was cooled to room temperature and held for use in the subsequent acylation.

A 500-mL, three-necked, round-bottomed flask equipped with mechanical stirring, a pressure-equalizing addition funnel, and a nitrogen-inlet adapter was charged with pyroglutamate salt **15** (20.0 g, 0.0336 mol, 1 equiv), 150 mL of EtOAc, 150 mL of water, and 16.5 g of $NaHCO_3$ (0.197 mol, 5.8 equiv). The suspension was mixed to dissolve the solids, and the solution of **16** (prepared above) was added dropwise over 5 min. After 30 min at room temperature, HPLC showed no unreacted starting material.

The layers were separated, and the organic layer was washed subsequently with 100 mL of 5% w/w aqueous $NaHCO_3$ and 100 mL of water and reduced to dryness in vacuo. The residual solid was dissolved in 100 mL of EtOAc and filtered (the collected solids were rinsed with EtOAc). The combined filtrates were reduced to a foam in vacuo. The foam was dissolved in 105 mL of EtOAc at 60 °C, and 105 mL of heptane at 60 °C was added. The solution was stirred at 60 °C briefly and cooled slowly to room temperature. After stirring at room temperature for 5 h, the product **2** was collected by filtration. The solid product was washed with 30 mL of 1:1 EtOAc/heptane and dried in vacuo at 70 °C for 60 h, affording 18.8 g (89% yield) of ABT-378 **2** as a colorless solid. Before crystallization crude **2** assayed as >93% pure by HPLC; after crystallization >99% purity was achieved.[20]

mp (EtOAc):[24] 124−127 °C. (uncorrected) IR: 3413, 3335, 3289, 3060, 2966, 1671, 1650, 1624, 1545, 1520, 1453, 1189, 701 cm^{-1}. ^1H NMR (300 MHz): δ 7.30−7.13 (m, 10H), 7.02−6.92 (m, 3H), 6.86 (v br s, 1H), 5.68 (br s, 1H), 4.25 (m, 1H), 4.19 (app d, J = 10 Hz, 2H), 4.19 (m, 2H), 3.78 (m, app d sept, 1H), 3.12 (m, 1H), 3.06 (m, 2H), 2.97 (d, J = 7.6 Hz, 2H), 2.88 (m, 1H), 2.81 (app ABX dd, J = 14, 5.2 Hz, 1H), 2.68 (app ABX, dd, J = 14, 9.5 Hz, 1H), 2.23 (m, 1H), 2.18 (s, 6H), 1.83 (s, 1H), 1.74 (m, 2H), 1.53 (m, 1H), 1.28 (m, 2H), 0.83 (app t, J = 7 Hz, 6H). ^{13}C NMR (75 MHz): δ 170.7, 168.8, 156.5, 154.2, 138.1, 138.0, 130.3, 129.3, 129.2, 129.0, 128.4, 128.2, 126.3, 126.0, 124.6, 70.2, 69.7, 63.1, 54.4, 48.7, 41.8, 41.1, 40.8, 40.0, 38.2, 25.4, 21.7, 19.6, 18.7, 16.1. MS (ESI) 629 (M + H)$^+$, 651 (M + Na)$^+$. Anal. Calcd for $C_{35}H_{48}N_4O_5$: C, 70.66; H, 7.69; N 8.91. Found: C, 70.26; H, 7.73; N 8.79. $[\alpha]_d^{20} = -22.85$ (c 0.4 MeOH)

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(24) Product crystallized in this manner contains approximately 2% residual ethyl acetate which cannot be removed by further drying.